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Evaluation of novel diagnostic tools for peste des petits ruminants virus in naturally infected goat herds

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SUMMARY

A survey was carried out in two goat herds during a single peste des petits ruminant (PPR) outbreak. Clinical examination showed that animals belonging to the West African Dwarf species had severe symptoms while those belonging to the West African long-legged species had mild symptoms. To confirm and to monitor the disease in each species, the study required specific monoclonal antibody-based diagnostic tools. An association of shedding of PPR virus (PPRV) and acute or mild clinical signs of the disease could be demonstrated by the rinderpest virus (RPV)/PPRV immunocapture ELISA assay. Between 85 and 100% of nasal secretions obtained from clinically diseased goats during the PPR outbreak reacted positively. Parallel serological surveillance for specific measurement of PPR antibodies revealed that between 34·4 and 88·5% of animals with no detectable virus were, however, able to seroconvert and therefore seemed to demonstrate that PPR subclinical infections do occur. Antibodies were shown to impair the RP heterologous vaccination. This evaluation offers new prospects for diagnosis and management of PPRV infection as well as for RPV control.

INTRODUCTION

Peste des petits ruminants (PPR) is an important pathogen of small ruminants in Africa, the Middle East and India. PPR virus (PPRV) mainly causes disease in sheep and goats, but it also causes subclinical infection in cattle with the development of a cross-neutralizing [1] and cross-protective [2, 3] humoral response against rinderpest (RP). The usual form of PPR is acute or subacute. PPR is characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis and pneumonia. These signs are not found together and non-clinical infections can be recorded for PPR as is the case for RP in cattle. It is not known if this is a result of infection with different strains of virus or a variation in the sensitivity of different breeds. Study of the epidemiology of PPR in small ruminants is further complicated by the use of a heterologous RP tissue, culture-attenuated vaccine for PPR prophylaxis in several African countries and in India.

The infection is usually confirmed by serology. Various techniques have been applied in the past, but only one, the virus neutralizing test (VNT) appears to distinguish between PPR and RPV serum antibodies [4]. The recent development of monoclonal antibody (mAb)-based ELISAs has allowed the rapid and simple differential diagnosis of RP and PPR. Competitive ELISA (C-ELISA) are used for specific detection of PPR or RP antibodies [5–7]. A definitive diagnosis is made by isolation of the virus, but this remains especially difficult for PPRV and other

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Morbilliviruses. As an alternative to isolation, antigen detection by immunocapture ELISA (ICE) is now also possible [8].

In this investigation we studied an outbreak of PPR in two breeds of goat. We found that ICE was reliable and useful for disease confirmation and monitoring under field conditions. The sensitivity and the specificity of the test were established in relation to the clinical diagnosis of the disease or the presence of specific antibodies. Serological surveillance for PPR antibodies revealed that PPR subclinical infections can occur in small ruminants. The value of RP vaccination during infection was also tested.

MATERIAL AND METHODS

Animals

The PPR outbreak occurred during the rainy season in a goat population housed in the Institut Sénégalais de Recherche Agricoles (ISRA) Dakar, Sénégal. For 2 weeks shed no. 1 housed 41 West African dwarf (WAD) goats originating from the South of the country. Thirty-one West African long-legged (WALL) goats were housed in shed no. 2. The groups lived in separated buildings and were always kept indoors. Nutritional and hygienic conditions were good.

When signs of the disease were observed, the affected animals and all the neighbouring small ruminants were given the heterologous RP tissue, culture-attenuated vaccine. This is a common practice in West African countries, with the aim of stopping the spread of the disease and limiting mortality.

Collection and processing of the samples

From the onset of the outbreak, goats were observed on a daily basis to identify animals suspected of being clinically diseased and to record mortality. They were then observed on a weekly basis over 4 weeks to monitor the excretion of the virus and seroconversion. During this time, nasal, ocular or oral secretions and serum were sampled. Samples of nasal secretion were obtained with cotton swabs, which were broken off in a sterile tube and kept on ice for transportation. Once at the laboratory, the sample was expressed from the cottonwool bud and tested with the ICE test. Serum samples were frozen at $-20\,^{\circ}C$ before testing. For some animals, post-mortem samples were also examined. They included spleen, lung and mesenteric lymph nodes. The autopsy specimens were stored at $-80\,^{\circ}C$ before further isolation.

Study design and statistics

To assess the reliability of the ICE test under field conditions, the assay response rate among disease and seroconversion groups was compared. Goats with acute or mild clinical signs were considered 'disease cases' and goats without either sign were 'non-cases'. All available secreta and tissue samples obtained from goats were tested by ICE, whereas the corresponding sera from the same animal and from the same week of sampling were submitted to PPR C-ELISA for the presence of antibodies against the nucleoprotein of the virus. Four patterns of disease and seroconversion were expected in goats: disease-positive/seroconversion-positive, disease-positive/seroconversion-negative, disease-negative/seroconversion-positive and disease-negative/seroconversion-negative. Estimates of sensitivity and specificity for ICE test were calculated [9] using the two different definitions of clinical PPR diagnosis: clinical disease status, and seroconversion status. Two group comparisons between diseased and antigen-positive animals were made using the χ^2 test at $\alpha = 0{\cdot}05$.

The same assay was used for the confirmation of the clinically suspected outbreak and the monitoring of the vaccinated animals. Each serum sample collected from goats was also processed for the presence of RP anti-haemagglutinin antibodies with H1 mAb C-ELISA [5] to evaluate the interaction of PPR infection on heterologous vaccination.

Virus isolation

The virus obtained during the epidemic was isolated from the lung of a diseased animal. Vero cells were inoculated with 100 μl of the specimen homogenate supernatant. Cells were maintained in Eagle's Minimum Essential Medium containing 2% fetal calf serum and antibiotics (40 μg gentamicin, 2·5 μg/ml amphotericin G, 100 μg/ml streptomycin sulphate). The medium was changed every 2 days and blind passages carried out once a week. The first cytopathic effects were observed 3 weeks after inoculation.

C-ELISA

The C-ELISAs for the detection of antibodies against RP (provided courtesy of J. Anderson) and PPR were based on similar protocols [5, 7]. Briefly, Nunc

Table 1. *Results of serological and virological survey for PPR disease conducted in two groups of goats*

Shed no.	Day	No. of animals		PPR serology (%)	Virology	
		Surviving	Diseased		ICE*	Virus isolation
1	1	25	25	24 (96)	21	+
	7	17	17	17 (100)	0	
	14	15	15	15 (100)	0	
	22	11	11	11 (100)	0	
2	1	31	1	9 (29)	2	−
	7	30	2	10 (33·3)	5	
	14	30	1	17 (56·7)	3	
	22	30	0	17 (56·7)	0	

* Nasal secretions.
ICE, immunocapture ELISA.

Maxisorb 96-well plates were used. They were coated with antigen diluted in PBS. After absorption and a cycle of three washes in 1:5 diluted PBS, the sera were incubated simultaneously with the specific mAb with blocking buffer containing Tween-20 and serum. The mAb was detected using rabbit anti-mouse enzyme-conjugated immunoglobulin. Reaction with the chromogen produced a colorimetric response, which was measured using a Titertek Multiskan Photometer (Labsystems, Helsinki, Finland) ELISA reader at 492 nm. Optical density (OD) values were converted to percentage inhibition values (PI) using the following formula:

$$PI = 100 - (\text{OD test}/\text{OD } 0\,\% \text{ control}) \times 100.$$

ICE test

The ICE test, produced as a kit, is based on a sandwich ELISA principle using couples of mAb. The method described by Libeau and others [8] was followed. In brief, Nunc Maxisorb plates were coated with anti-RP and PPR cross-reactive mAb diluted in PBS as a capture antibody. PPR or RP antigens were detected with specific biotinylated mAbs in samples incubated in duplicate on plates. The conjugate was streptavidin peroxidase. Reaction with the chromogen mixture, hydrogen peroxide and orthophenylene diamine, produced a colorimetric response signifying a positive test.

RESULTS

Animals

Records for this study began with the onset of the disease. The disease first occurred in shed no. 1 where WAD goats originating from South Senegal were introduced for quarantine. They showed acute signs of PPR: fever, cough, respiratory distress, visible mouth lesions, mucopurulent oral and nasal discharges, diarrhoea and rapid death. Because of their high suseceptibility to PPRV, 25 animals out of 41 were recorded with disease on the first day of the investigation. In the same week, the WALL goats from shed no. 2 began to display clinical signs but in a very mild form. All the animals in this group, except one, survived. These goats had been living inside the laboratory enclosure for several months without any problem until the WAD group introduced the disease.

Survey for PPR infection in two goat species

To evaluate the influence of PPR infection on the goat species, disease and serological survey as well as antigen detection and virus isolation were conducted on samples taken from the WAD and WALL goats at intervals after natural infection. These results are summarized in Table 1. In shed no. 1, in which the WAD goats were kept, PPRV was detected from swabs by ICE on day 1 in 21 out of 25 animals which had clinical signs of PPR infection. However, none of them excreted the virus for more than 1 week. A high prevalence of antibodies against PPRV was also detected by PPR C-ELISA (96–100%). Nevertheless, 14 animals died demonstrating that elimination of PPRV does not prevent fatal after-effects, such as pneumonia [10]. From the autopsy specimen collected from one WAD goat (the lung) it was possible to isolate the virus after four blind passages on Vero cells. The strain was later shown to belong to lineage I, as are most strains from western Africa (data not shown).

Table 2. *Disease status and PPR C-ELISA results from WALL goats excreting virus*

Goat no.	Disease status	ICE*				PPR C-ELISA			
		Day 1	Day 7	Day 14	Day 22	Day 1	Day 7	Day 14	Day 22
563	ND	+	−	−	−	+	+	+	+
564	ND	−	−	+	−	−	−	+	+
568	ND	−	+	−	−	−	−	+	+
574	ND	−	+	−	−	−	+	+	+
578	D	−	+D	+	−	−	−D	−	−
584	ND	−	+	−	−	−	+	+	+
597	D	−	−	+D	−	−	−	+D	+
599	ND	−	+	−	−	−	−	+	+
600	D	+D	+†	d	d	+D	d	d	d

ICE, immunocapture ELISA.
ND, not diseased; D, diseased; +, animal detected positive; d, died.
* Detection of nucleoprotein in nasal secretions.
† Post-mortem sample.

Table 3. *Serological results of the interaction between PPR infection and RP vaccination*

Shed no.	Day	No. of animals surviving	Rinderpest seroconversion		
			Total (%)	With PPR antibodies	Without PPR antibodies
1	1	25	0 (0)	0/24	0/1
	7	17	0 (0)	0/17	0/0
	14	15	4 (26·7)	4/15	0/0
	22	11	4 (36·4)	4/11	0/0
2	1	31	0 (0)	0/9	0/22
	7	30	0 (0)	0/10	0/20
	14	30	5 (16·7)	2/17	3/13
	22	30	11 (36·7)	3/17	8/13

In shed no. 2, only three WALL goats displayed some mild clinical signs during the study. One died in the second week but the others recovered. Virus identification by ICE provided confirmation of the presence of PPRV in nine animals during the 4 weeks of examination although only three of them showed clinical signs. PPRV excretion was detectable in two animals on the first day of examination, and most of the remainder during the second week. Antigen was not detectable after 3 weeks of infection. The serological survey of the whole group also demonstrated a low, but substantial response against PPRV. However, one of the animals, which displayed clinical signs, did not seroconvert (no. 578) (see Table 2). The overall prevalence of antibodies to PPRV reached 56·7% (17/30) by the end of the experiment, suggesting that there were many more infected animals than those exhibiting clinical signs.

PPR infection and heterologous vaccination

Serum samples from goats were also tested for antibodies against RPV by C-ELISA, and these results are shown in Table 3. Seroconversion to RP occurred 2 weeks after vaccination as has been described previously [1, 11]. At the end of the experiment (day 22) four WAD goats (36·4%) and 11 WALL goats (36·7%) had seroconverted. As the animals were

Table 4. *ICE ELISA test results on samples obtained from goats during the PPR outbreak*

	Positive ICE response/total	
	Confirmation	Monitoring
Results of disease status and seroconversion		
Disease positive, seroconversion positive	21/25	2/2
Disease positive, seroconversion negative	1/1	1/1
Disease negative, seroconversion positive	0/7	4/50
Disease negative, seroconversion negative	1/23	3/68
Total	23/56	10/121
	$\chi^2=7{\cdot}813$, $P=0{\cdot}005$	$\chi^2=0{\cdot}019$, $P=0{\cdot}891$
Sensitivity		
vs. disease	22/26 (84·6)	3/3 (100)
vs. seroconversion	21/32 (65·6)	6/52 (11·5)
Specificity		
vs. disease	29/30 (96·7)	111/118 (94·1)
vs. seroconversion	22/23 (95·7)	65/69 (94·2)

ICE, immunocapture ELISA.

immunized with RP vaccine during the outbreak of PPR, the development of the vaccinal response in individuals was also a function of previous immunity against PPR. Among goats which had PPR antibodies prior to vaccination, 18·8% (6/32) and 25·0% (7/28) seroconverted to RP on days 14 and 22 respectively. In contrast, among goats which had no PPR antibodies, 23·1% (3/13) and 61·5% (8/13) seroconverted on the same day after vaccination.

Sensitivity and specificity of ICE

The ICE test was used to confirm cases of PPR suspected on clinical grounds. At the time of discovery of the disease, 26 out of 56 animals were sick, and 33 already had PPR antibodies (most from shed no. 1; Table 1). There were 23 ICE-positive samples out of 56 tested, 22 were from diseased animals and one from a healthy animal. The association of disease and antigen detection was highly significant ($P=0{\cdot}005$). Defining clinical disease as the reference for the clinical diagnosis of PPR, the ICE response rate (Table 4) reached a sensitivity of 84·6% and a specificity of 96·7%. Compared to seroconversion, the ICE test had only a positive predictive value of 65·6% suggesting the 34·4% seropositive animals were not detected by ICE. The negative predictive value, however, reached 91·7%.

The ICE test was also used to monitor the vaccinated WALL goats over 4 weeks. In this group, only 3 out of 31 animals were sick, and many seroconverted without being sick (Tables 1 and 2). Overall, there were 10 ICE-positive results out of 121 samples tested: three were from diseased animals and seven from healthy animals. No significant association could be made between shedding of antigen and disease ($P>0{\cdot}05$). When compared to disease or seroconversion (Table 4), the sensitivity of the ICE test was 100 and 11·5% respectively, while the specificity was 94·1 and 94·2% respectively. In this group, the test was positive for every clinical case, but ICE had a poor positive predictive value for seropositive animals: 88·5% of animals negative by ICE seroconverted, and most of these animals did not show any clinical signs of disease. However, the test had a good negative predictive value (94·2%) for seronegative animals.

Cross-reactivity of the ICE test with other antigens, especially RPV, was not expected [8]. However a double-positive sample (nasal swab) was obtained with the ICE test for animal no. 597 on day 14.

DISCUSSION

PPR has major economic consequences for sheep and goat farming, owing to the highly contagious nature and the rapid spread of the disease. Around 800 million animals are estimated to be susceptible in countries which report the presence of the disease [12]. Indeed PPR, originally thought to be confined to West Africa, is widely distributed in sub-Saharan

Africa, the Middle East and the Indian subcontinent [4, 13–19]. Serological surveys have shown high prevalences of antibodies against PPRV in small ruminants in African countries [20, 21], but only few studies of the pathogenicity of PPRV in goats under natural or experimental conditions have been published thus far [15, 17, 19].

From observing a PPR outbreak in two different breeds of goats, we found that the acute form of the disease affected WAD goats while WALL goats developed only the milder form. This difference in sensitivity to disease seems most likely to be related to the breed and not to the virus. PPR viruses from Africa and Asia are found to group in four distinct lineages [18] (A. Diallo, personal communication), and WAD goats have recently been shown to be extremely sensitive to PPRV whatever the virus lineage involved (E. Couacy, personal communication). Viruses isolated from western Africa, including the isolate described in this report, form lineage I.

During the PPR outbreak, good antibody responses against PPRV were provoked in both goat breeds. However the value of vaccination during the risk of infection, in this case with the heterologous vaccine, was tested. A poor but effective response (37%) against RP vaccine was obtained with a zero to moderate (31·6%) increase of immunity coverage defined by the combination of antibodies against both RPV and PPRV. A total of 75% of the animals, which had developed cross-neutralizing antibodies against PPRV, did not respond to RP vaccine. This supports data from a previous field trial where the unusually low response to RP vaccination of cattle, sheep and goats was explained by the presence of antibodies against PPRV before vaccination [20]. However, in this study almost all animals (96–100%) which failed to respond to RP vaccine were found to be PPR positive. Kulkarni and others [17] also reported that for goats, the protection conferred by RP tissue culture vaccine to a population at risk might only be partial. This incomplete coverage of the population, or possibly faulty vaccination procedures, resulted in PPR outbreaks within a few months of RP vaccination. However, heterologous vaccination is known to protect small ruminants against PPR for at least 12 months [3]. Although described here for small ruminants, these observations can be transposed to cattle. Indeed, possible experimental transmission of PPRV from sheep and goats to cattle [22] as well as serological evidence of the circulation of the virus [20, 23], need to be taken into consideration when applying and evaluating the RP control programmes in PPR endemic areas.

An association of shedding of PPRV and acute or mild clinical signs of the disease was seen in the present study. The results obtained on 56 animals tested during the outbreak showed that ICE ELISA, an antigen detection test for the nucleoprotein of PPRV and RPV, enabled early and direct demonstration of the presence of PPRV in nasal secretions and in post-mortem specimens: between 85 and 100% of specimens from clinically infected goats were positive in this assay. For RP the severity of the clinical signs is correlated with the abundance of viral antigen in lymphatic organs and the mucosae along the digestive tract. It is likely that the same is true for PPRV. However, even in animals that had only mild disease, the rate of antigen positivity was quite high, demonstrating the good sensitivity of the ICE test. For the WALL goat population, a peak of excretion could be detected at 1 week post-infection, before the rise of antibodies at 14 days.

The poor sensitivity of the test (11·5%) compared to seroconversion, however, suggests that during the recovery period the infected animals are unlikely to be detected by antigen detection procedures.

In this study, ICE did not usually detect the RPV vaccine in secretions. This is probably because in losing its virulence, the RPV vaccine strain loses its epithelial tropism [24, 25]. Therefore, it is interesting to speculate that the one RPV-positive animal arose from RPV-infected lymphoid cells infiltrating the epithelium. Indeed the PPR-infected animal no. 597 was showing signs of the disease.

The relative specificity estimates of the test suggested that 3·3–8·3% of goats with no serological or clinical sign of the disease would have detectable amounts of virus in their nasal secretions. It is believed that these subclinically infected animals can initiate or perpetuate the infection in susceptible animals when introduced in a new herd. For these reasons, disease control procedures must be used on infected as well as on suspected herds.

Epidemiological interactions of PPR and RP infections and/or vaccination in domestic ruminants occurring in Africa and Asia need to be better understood. The routine application of direct and individual antigen detection using an immunocapture test accompanied by the serological confirmation offers new prospects for controlling PPRV.

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